

AMENDMENTS TO THE WRITTEN DESCRIPTION:

1. Please replace the paragraph beginning at page 7, line 12, with the following rewritten paragraph:

E² - The use of chelating agents for radiolabeling peptides, and methods for labeling peptides with Tc-99m are known in the prior art and are disclosed *inter alia*, in co-pending U.S. Patent Applications Serial Nos. 07/653,012 08/263,758 (now U.S. Patent No. 5,654,272), 07/807,062 (now U.S. Patent No. 5,443,815), 07/871,282 (now U.S. Patent No. 5,965,107), 07/886,752 08/273,274 (now U.S. Patent No. 5,849,260), 07/893,981 now U.S. Patent No. 5,508,020, 07/955,466 08/361,864 (now U.S. Patent No. 5,977,064), 08/019,864 (now U.S. Patent No. 5,552,525), and 08/073,577 (now U.S. Patent No. 5,561,220), and radiolabeled peptides for use as scintigraphic imaging agents for imaging thrombi are known in the prior art and are disclosed in co-pending U.S. Patent Applications Serial Nos. 07/886,752 08/273,274 (now U.S. Patent No. 5,849,260), 07/893,981 (now U.S. Patent No. 5,508,020) and 08/044,825 08/439,905 (now U.S. Patent No. 5,645,815) which are hereby incorporated by reference.--

2. Please replace the paragraph beginning at page 9, line 5, with the following rewritten paragraph:

B - In one embodiment of the reagents of the invention, the radiolabel-complexing moiety has a formula that is -(amino acid)¹-(amino acid)²-(amino thiol) or (mercaptocarboxylic acid)-(amino acid)¹-(amino acid)²-, wherein (amino acid)¹ and (amino acid)² are each independently any ~~naturally occurring~~ naturally occurring, modified, substituted or altered primary α - or β -amino acid; (amino thiol) is selected

E3
~~from~~ from the group consisting of cysteine, isocysteine, homocysteine, penicillamine, 2-mercaptoethylamine, and 3-mercaptopropylamine; and (mercaptocarboxylic acid) is selected from the group consisting of cysteine, isocysteine, homocysteine, penicillamine, 2-mercaptoacetic acid, and 3-mercaptopropionic acid. --

3. Please replace the paragraph beginning at page 9, line 13, with the following rewritten paragraph:

E4
-- In preferred embodiments, the Tc-99m complexing moiety of the invention
~~comprise~~ comprises moieties having the formulae -Gly-Gly-Cys- or Cys-Gly-Gly-. --

4. Please replace the ~~paragraph~~ list beginning at page 12, line 3, with the following rewritten ~~paragraph~~ list:

E5
cyclo(N-methyl)FYW_DKV,Hcy.(CH₂CO.GGC.amide)
cyclo(N-methyl)FYW_DKV,Hcy.(CH₂CO.GGCK.amide)
cyclo(N-methyl)FYW_DKV,Hcy.(CH₂CO.GGCR.amide)
cyclo(N-methyl)FYW_DKV,Hcy.(CH₂CO.GGCRD.amide)
cyclo(N-methyl)FYW_DKV,Hcy.(CH₂CO.GGCRK.amide)
cyclo(N-methyl)FYW_DKV,Hcy.(CH₂CO.GGCRR.amide)
cyclo(N-methyl)FYW_DKV,Hcy.(CH₂CO.GGCKK.amide)
cyclo(N-methyl)FYW_DKV,Hcy.(CH₂CO.GGCKKK.amide)
cyclo(N-methyl)FYW_DKV,Hcy.(CH₂CO.GGC.Orn.amide)
cyclo(N-methyl)FYW_DKV,Hcy.(CH₂CO.GGCKDK.amide)
cyclo(N-methyl)FYW_DKV,Hcy.(CH₂CO.GGC.Orn.D.Orn.amide)

cyclo(N-methyl)FYW_DKV,Hcy.(CH₂CO.GGC.Orn.D.amide)

cyclo(N-methyl)FYW_DKV,Hcy.(CH₂CO.KKC.amide)

cyclo(N-methyl)FYW_DKV,Hcy.(CH₂CO.KRC.amide)

cyclo(N-methyl)FYW_DKV,Hcy.(CH₂CO.RRC.amide)

cyclo(N-methyl)FYW_DKV,Hcy.(CH₂CO.KKCK.amide)

cyclo(N-methyl)FYW_DKV,Hcy.(CH₂CO.GRCK.amide)

cyclo(N-methyl)FYW_DKV,Hcy.(CH₂CO.GKCR.amide)

CH₂CO.Y_D.Apc.GDCGGC_{Acm}GC_{Acm}GGC.amide

CH₂CO.Y_D.Apc.GDCGGC_{Acm}GC_{Acm}GGCG.amide

CH₂CO.Y_D.Apc.GDCGGSSGGCG.amide

CH₂CO.Y_D.Apc.GDCGGCG.amide

GRGDGGC (SEQ ID NO: 1)

GLFCGC.amide (SEQ ID NO: 2)

GRGDGGGGC (SEQ ID NO: 3)

F_DFYW_DKTFTGGC.amide

acetyl.CGgy.[(CH₂)₄-piperidine] (SEQ ID NO: 4)

5. Please replace the paragraph beginning at page 14, line 2, with the following rewritten paragraph:

--Radiolabel complexing moieties of the invention may be introduced into the target specific peptide during peptide synthesis. The radiolabel complexing moiety can be introduced into the peptide to comprise the amino- or carboxyl-terminus of the peptide. In addition, ~~radiolabel complexing~~ radiolabel complexing moieties may be covalently linked to the groups comprising the ~~sidechains~~ sidechains of amino acids, for example, the ϵ -amino group of lysine to give, for example, α N(Fmoc)-Lys- ϵ N[Gly-Gly-

Cys], which may be incorporated at any position in the peptide chain. This sequence is particularly advantageous as it affords an easy mode of incorporation into the target binding peptide. This invention provides for the incorporation of these chelators into virtually any peptide, resulting in a radiolabeled peptide covalently linked to a Tc-99m complexing moiety. --

6. Please replace the table on page 19 with the following rewritten table:

TABLE 1

| <u>Peptides</u> | <u>FABMS</u> <u>MH⁺</u> | <u>Radiochemical</u> <u>Yield (%)[*]</u> | <u>HPLC</u> <u>R_T(min)^{**}</u> |
|--|---------------------------------------|--|---|
| <i>cyclo(N-methyl)FYW_DKV,Hcy</i> .(CH ₂ CO.GGC.amide) | 1129 | 98 ⁴ | 15.1, 17.2 |
| <i>cyclo(N-methyl)FYW_DKV,Hcy</i> .(CH ₂ CO.GGCK.amide) | 1258 | 99 ⁴ | 15.0 |
| <i>cyclo(N-methyl)FYW_DKV,Hcy</i> .(CH ₂ CO.GGCR.amide) | 1285 | 99 ³ | 15.1 |
| <i>cyclo(N-methyl)FYW_DKV,Hcy</i> .(CH ₂ CO.GGCKK.amide) | 1386 | N.D. | N.D. |
| <i>cyclo(N-methyl)FYW_DKV,Hcy</i> .(CH ₂ CO.GGC.Orn.amide) | 1244 | 98 ⁶ | 7.0 ¹ |
| <u>CH₂CO.Y_D.Apc.GDCGGC_{Acm}GC_{Acm}GGC.amide</u> | 1393 | 97 ⁴ | 11.4 ³ |
| <u>CH₂CO.Y_D.Apc.GDCGGSSGGCG.amide</u> | 1219 | N.D. | N.D. |
| <u>CH₂CO.Y_D.Apc.GDCGGCG.amide</u> | 930 | 99 | 11.3 ³ |
| <u>CH₂CO.Y_D.Apc.GDCGGC_{Acm}GC_{Acm}GGCG.amide</u> | 1450 ⁷ | N.D. | N.D. |
| F _D FYW _D KTFTGGC.amide | 1356 | 99 ⁴ | 15.2, 16.2 |
| GRGDGGC (<u>SEQ ID NO: 1</u>) | 769 | 98 ² | 13.0, 13.6, 14.7 ³ |
| GRGDGGGGC (<u>SEQ ID NO: 3</u>) | 735 | 100 ¹ | 14.9, 15.1, 15.4 ² |

7. Please replace the paragraph beginning at page 20, line 16, with the following rewritten paragraph:

E⁸ -- Single-letter abbreviations for amino acids can be found in G. Zubay, *Biochemistry* (2d. 2d ed.), 1988 (~~MacMillan~~ Macmillan Publishing: New York) p.33. Underlining indicates the formation of an amide or a thiol linkage between the linked amino acids of derivative groups. Ac_m is acetamidomethyl; Orn is ornithine; F_D is D-phenylalanine; Y_D is D-tyrosine; W_D is D-tryptophan; Apc = ~~L-[S-(3-aminopropyl)cysteine~~ L-[S-(3-aminopropyl)]-cysteine; and Hcy is homocysteine. --

8. Please replace the paragraph beginning at page 20, line 25, with the following rewritten paragraph:

E⁹
- Platelet aggregation studies were performed essentially as described by Zucker (1989, Methods in Enzymol. 169:117-133). Briefly, platelet aggregation was assayed with or without putative platelet aggregation inhibitory compounds using fresh human platelet-rich plasma, comprising 300,000 platelets per microlitre. Platelet aggregation was induced by the addition of a solution of adenosine diphosphate to a final concentration of 10 to 15 micromolar, and the extent of platelet aggregation monitored using a Bio/Data aggregometer (Bio/Data Corp. Horsham, PA). The concentrations of platelet aggregation inhibitory compounds used were varied from 0.1 to 500 µg/mL. The concentration of inhibitor that reduced the extent of platelet aggregation by 50% (defined as the IC₅₀) was determined from plots of inhibitor concentration *versus* extent of platelet aggregation. An inhibition curve for peptide RGDS (SEQ ID NO:5) was determined for each batch of platelets tested as a positive control.

9. Please replace the paragraph beginning at page 21, line 1, with the following rewritten paragraph:

E¹⁰
- The results of these experiments are shown in Table II. In Table II, the compounds are as follow: (RGDS (SEQ ID NO: 5) is given as a positive control)

TABLE II

| | | |
|--------|---|----------------------------|
| P686 = | <u>CH₂CO.Y_D.Apc.GDCGGSSGGCG.amide</u> | IC ₅₀ = 0.34 µM |
| P246 = | <u>CH₂CO.Y_D.Apc.GDCGGC_{Ac}GC_{Ac}GGC.amide</u> | IC ₅₀ = 0.63 µM |

P645 = $\text{CH}_2\text{CO.Y}_D\text{.Amp.GDCGGC}_{\text{Acm}}\text{GC}_{\text{Acm}}\text{GGC.amide}$ $\text{IC}_{50} = 0.67\mu\text{M}$
P665 = $\text{CH}_2\text{CO.Y}_D\text{.Apc.GDCGGSSGGCG.amide}$ $\text{IC}_{50} = 0.80\mu\text{M}$
P676 = $\text{CH}_2\text{CO.Y}_D\text{.Apc.GDCGGC}_{\text{Acm}}\text{GC}_{\text{Acm}}\text{GGCG.amide}$ $\text{IC}_{50} = 0.97\mu\text{M}$

E¹⁰
(Single-letter abbreviations for amino acids can be found in G. Zubay, *Biochemistry* (2d. ed.), 1988 (MacMillan Macmillan Publishing: New York) p.33 as discussed in the Legend of Table I; Acm = acetamidomethyl; Amp = 4-amidinophenylalanine; Apc = L-[S-(3-aminopropyl)cysteine L-[S-(3-aminopropyl)]-cysteine; Y_D = D-tyrosine] --

10. Please replace the paragraph beginning at page 23, line 3, with the following rewritten paragraph:

E¹¹
--Tc-99m labeled P199 (*mercaptoacetyl*GGGRALVDTLKFVTQAE~~GAK~~.amide (SEQ ID NO: 6)) was prepared as described above. Approximately 1000 μg of peptide was labeled with 100-200mCi of Tc-99m and prepared in unit doses of 5-10mCi (12.5-20.0 μg /rabbit; 6-7 μg /kg) in 0.5-2mL volume doses. Adult rabbits were dosed with Tc-99m labeled peptide intravenously in a lateral ear vein by slow bolus infusion (approximately 0.1mL/min). A gamma camera fitted with a pin-hole collimator (5mm aperture) and energy window set for Tc-99m and programmed to accumulate 500,000 counts or scan for a desired time. Shortly before imaging, animals were anesthetized with a mixture of ketamine and xylazine (5:1, 1mL/kg intramuscularly). --

11. Please replace the paragraph beginning at page 24, line 17, with the following rewritten paragraph:

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Serial No. : 08/236,402
Filed : May 2, 1994
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Attorney's Docket No.: 09744-006001 / DITI 107

E12
-- New Zealand White (NZW) rabbits of both sexes and weighing 2-3 kg
are ~~innoculated~~ inoculated intramuscularly in the left calf with a potent strain of
Escherichia coli. After 24 hours, the animals are sedated by intramuscular injection of
ketamine and xylazine and then injected with Tc-99m labeled peptide (2-10mCi, \leq
150 μ g). The animals are then positioned supine in the field of view of a gamma
~~cammera~~ camera (LEAP collimator/photopeaked for Tc-99m) to be imaged. The animals
are imaged over the first hour post-injection, and then at approximately 1 hour intervals
for the next 3 hours. Animals are allowed to recover between image ~~acquistions~~
acquisitions and re-anesthetized as needed. --